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ASSESSMENT OF EFFECTS ON PLANT GROWTH BY TREATED LANDFILL LEACHATE

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ABSTRACT

Groundwater incorporates a high risk of being impure around the areas close to lowlands because of the potential pollution source of landfill leachate. Leachate can also be used via bioremediation as a fertilizer for plant growth. In bioremediation process, microorganism use the contaminants as nutrient or energy source. From the results it can be concluded that the leachate sample collected from Anand had lower amount of COD, BOD, nitrate, nitrite and ammoniacal nitrogen compared to the leachate collected from AMC. Thus, the leachate from AMC had higher content of heavy metals. Biosorption assay was performed to treat the leachate samples which relatively showed decreased amount of heavy metals after the treatment of leachate.

Keywords: leachate, COD, BOD, TS, TSS

INTRODUCTION

Groundwater incorporates a high risk of being impure around the areas close to lowlands because of the potential pollution source of landfill leachate. Hence, the study of landfill leachate has become prominant in recent years. A landfillis a site for the disposal of waste materials by burial and is that the oldest form of waste treatment **[1]**.

The main purpose of landfilling is to stabilize the waste. Landfilling of municipal waste is still a very important issue of the waste management system in

the world. Leachate, defined as water that has percolated through the wastes(rainwater or groundwater seepage), which is a main source of soil and groundwater contamination **[2]**. When refuse is buried in landfill, a complex series of biological and chemical reactions occurs due to the decomposition of refuse. The common type of thelandfills receives a mixture of municipal, commercial, and mixed industrial waste, but excludes significant amounts of targeted specific chemicals waste. Landfill leachate is also characterized as water primarily based on aqueous solution of four groups of pollutants; dissolved organic matter, inorganic macrocomponent, significant heavy metals and xenobiotic organic compounds **[3]**. The leachate produced by such landfills have deteriorated the ground water quality and thus, decreased the availability of drinking water.

Leachate is defined as 'the liquid that drains or leaches from dumping sites of waste or it is generated by excess rainwater percolating through the waste layers in a landfill'. Precipitation and pressing of waste is major factor for production of leachate. Waste contains both dissolved and suspended material. Landfill leachate is generally dark colour liquid, bad odour which contain both organic and inorganic matter. Leachate mainly consists of an aqueous solution and this solution contains 4 groups of pollutants : dissolved organic matter (Volatile Fatty Acid and certain refractory organic matter such as Humic substance), macro inorganic compounds (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, NH^{+}_{4}, Fe^{2+}, HCO_3^{-}), heavy metals (Cd^{2+}, Cr^{3+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, and Zn^{2+}) and xenobiotic organic compounds originating from chemicals and domestic residues and also at low concentration (aromatic hydrocarbons, Phenols and Pesticides) are present **[4]**.

However, landfill leachate varies widely in composition depending on age of landfill and type of waste. Once the landfill stops is filled with the waste then final cover is placed over the landfill and decomposition of wastes will take place, while the leachate production decreases. Proper collection, treatment and disposal of leachate are required to boost better environment and healthful condition for quality life **[5]**.

Leachate is the undesirable product of the landfills. It contain metals like (Ag, Cd, Pb, Zn, Cr, Cu, Fe, K and Hg). Some metals serves as macronutrient and some are non-essential. They are potentially toxic to human and surrounding if it is present beyond the permissible range. This contributes to bioremediation, 'use biological organisms to solve an environment problem such as contaminated soil or groundwater pollution'. Leachate can also be used via bioremediation as a fertilizer for plant growth. In

bioremediation process, microorganism use the contaminants as nutrient or energy source [**6-9]**. Bioremediation is consider as safer and less expensive method compared to incineration.

MATERIALS AND METHODS

All glass and plastic equipments and specific chemicals were procured locally.

Collection of samples from the landfill site

The samples were collected from the two different sites; one was a landfill site, Gyaspur (Ahmedabad) and the other one was sewage site, Anand. The samples were collected phosphate free plastic bottle. Without disturbing the sample, sample was sealed and packed with aluminium foil along with plastic bag to avoid the contamination and leakage of sample. All the parameters were evaluated by the standard protocol as mentioned in APHA, AWWA, and WEF, 2005. Standard methods for the examination of water and waste water **[10]**.

Characterization of Landfill leachates

Colour, appearance and odour of both samples were evaluated physically. The odour was described as desirable and undesirable and further detailed method described in chemical characterization. The appearance described with respect of the type of solid particle present and in which form it was found.

Chemical Characterization [10]

Biochemical Oxygen Demand (BOD) by dilution method

It is bioassay procedure. Oxygen present in the sample oxidizes the divalent manganese to its higher valency, which precipitates as brown-hydrated oxides after addition of NaOH and KI. Upon acidification, manganese revert to divalent states and liberate iodine from KI. The liberated iodine was titrated against $Na_2S_2O_3$ using starch as indicator.

Measurement of Chemical Oxygen Demand (COD):

Chemicals Oxygen Demand (COD) test determines the oxygen required for the chemical oxidation of most organic matter and oxidizable inorganic substances with help of the strong oxidants. The organic matter and oxidisable inorganic substances present in wastewater get oxidized completely by standard potassium dichromate ($K_2Cr_2O_7$) in presence of H_2SO_4 to produce CO_2 + H_2O. The excess K2Cr2O7 was titrated with Ferrous Ammonium Sulphate (FAS). By using following formula:

$$\textbf{COD (mg/L)} = \frac{[(B-A)*N*8*1000]}{\text{ml of the sample}}$$

Where:

N= Normality of FAS (0.25N)

8= Miliequivalent weight of oxygen

B= ml of FAS used for blank

A= ml of FAS use for sample

Total Organic Carbon (TOC)

Organic carbon present in soil organic fraction, which consist cells microorganism, plants and animal residue. At various stages of decomposition stable humus synthesized from residue and highly carbonized compound such as charcoal, graphite and coal. The organic matter in leachate sample gets oxidized by $K_2Cr_2O_7$ + conc.H_2SO_4. The excess $K_2Cr_2O_7$ is determine by back titration with FAS.

Organic carbon (%) = $\frac{10\%(B-T)}{B} \times 0.003 \times \frac{100}{S}$

Where,

B= volume of FAS required for blank, ml

T = volume of FAS required in sample, ml

S = weight of sample in g.

Estimation of Total Nitrogen

Nitrate was estimated by Brucine sulphate method which is based upon the reaction of nitrate ion with Brucine sulphate in 13 N H_2SO_4 solutions at 100˚ C temperature. Nitrite was meseaured by NEDA method which shows that under acidic condition nitrite ion as nitrous acid reacts with sulphanilamide to form diazonium salt which combine with N(1-napthyl ethylenediamine dihydrochloride (NEDA) to form bright pink colour azo dye and the colour reduced is directly proportional to amount of nitrite present in the sample. Similarly, ammoniacal nitrogen is estimated by sodium nitroprusside method **[10]**.

For the Estimation of heavy metal like Chromium, iron etc and Total Solids (TS), Total Dissolved Solids (TSS), Total Dissolved Solids (TDS) were estimated by following the standard protocol as mentioned in AWWA **[10]**.

Bioremediation of sample [11]

Removals of heavy metals from contaminated leachate with help of bacteria were isolated from contaminated soil and leachate sample. Identification was done with help of Gram's staining followed by biochemical test. The test which are carried out by using **Voges-Proskauer (V-P) Test**

Isolation of bacteria from soil sample

Bacteria were isolated from waste soil sample which was collected from the landfills site. Prepare soil suspension by making different dilution [10^{-1} to 10^{-6}]. Each dilution was spread with help of pour plate method on labelled Petri-plates containing 20ml BHM medium + 50ppm of heavy metals solution (Fe, Cr and Pb). The plates were kept for incubation at 37˚C for 24hrs or until the colonies were observed. After incubation, different colonial morphologies of bacteria were chosen and purified by successive re-streaking (four flame method). The pure bacterial cultures obtained were maintained on Nutrient Agar

slants and can be used in further experiment.

Characterization of bacteria

There are various methods for characterization of bacteria. Traditionally an observational and biochemical approach has been used.

Colony characterization

The bacterial colonies were characterized on the basis of their size, shape, texture, margin, opacity and pigmentation. It is usually done by observing the colony with naked eye. Colony morphology is one of the means in identifying bacterial species [**12]**.

Gram staining

Thin smear was prepared on glass slide from fresh bacterial growth. Primary stain: smear was stained with crystal violet for 1 min. Then smear was flooded with gram's iodide and allowed to remain for 1min. The slide was washed with tap water for 2 seconds. Then smear is decolorized with 95% ethyl alcohol for 10 seconds. Counter stain: smear was stain with saffranin and allowed it to stain for 1min. The smear was washed with tap water, blotted and air dried. Smear was examined under 100x oil immersion lens. Morphologies and staining pattern observed and recorded.

Biochemical studies of bacteria was conducted by using Methyl red (M-R) Test, **Voges-Proskauer (V-P) Test, Citrate Utilization Test, Indol production Test, Catalase Test [13-15]**

Biosorption assay [16]

Prepare BHM broth in 250 ml conical flask. The broth medium was sterilized by autoclaving. After autoclaving distribute 20 ml broth in labelled sugar tubes. Add isolated pure colony in to BHM broth. Broth was incubated on shaker under constant agitation at 100rpm. After incubation turbidity was observed in to different broths. Next day prepare new BHM broth in 500ml conical flask. The broth medium was sterilized by autoclaving. After autoclaving distribute 100ml broth in labelled 250 ml conical flask. Each flask contain 100ml broth + 2ml of bacterial suspension and different concentration of leachate (2 ml 4 ml and 6 ml). Kept the flasks under agitation in rotary shaker for 48-72 hours or till broth become turbid

Pot studies [17-19]

To check what was the effect of leachate on plants and whether leachate can be used or not after its treatment with microbes. Pot studies were carried out to see its effect on plants with different concentration of leachate and treated leachate. Three plants were selected, all three plants grow quickly at same time but some are sensitive against 100% leachate concentration. The plants were *Solanum lycopersicum*, *Cucumis sativus* and Capsicum frutescent. Different

concentration of 10%, 20%, 40%, 60% and 100% of both samples were made by adding normal tap water. A control or 0% concentration and pot containing commercially available N, P, and K were also maintained. The seedlings were planted accordingly and were given all necessary conditions for growth. The plants were observed on daily basis. After 15 days plants were thoroughly studied by uprooting them from soil. The growth and other characteristic were than compared and noted.

RESULT AND DISCUSSIONS

The results were recorded as follows:

Physical Characterization

The sample collected from Gyaspur landfill site was dark brown liquid while the sample from Anand site was greyish liquid. Both liquid sample contained mixed suspended tiny particle and residues of plant debris.

The odour of untreated sample was unfavourable.

Presence of heavy metals and industrial and other chemicals effluents contributes the blackish colour to the sample. While dumping of the various types of waste especially household and community derived waste gives typical odour to the sample.

The physical characteristics of samples were tabulated in **Table 1**.

Sample 1: AMC; **Sample 2**: Anand site

Chemical Characterization

The pH of both samples were ranging from 7-7.4 explaining the nature of leachate and favourable for the growth of microorganism.

The BOD is the degree of the pollution, higher values of BOD resembles to more polluted sample. BOD values ranged from >1500 ppm. Both sample was highly polluted.

The COD values ranged from > 20000ppm.The sample contain higher amount of organic matter.

The TSS values were under the normal range and showing that suspended solids are present in a measurement amount.

The TDS indicates the general nature of water quality or salinity. The range of TDS was high which may be because of excessive pollution of various waste material.

Turbidity was checked with help of turbidometer. Sample were slightly turbid ranging from 20-70 NTU.

Nitrogen was present in high amount in form of ammonical nitrogen. Nitrate and Nitrite present in considerable amount.

This indicates that wastes contain many pollutants and it should not disposed off directly to water bodies.

The chemical characteristics of samples were tabulated in **Table 2**.

Isolation, Screening and identification of bacterial culture

As evidenced in **Figure 3a to 3c**; different types of colonies were observed on the plates. Colonies which shows different characteristic were transferred onto new plates. They were characterized morphologically and on the basis of biochemical test.

Morphological and Cultural Characteristic of bacterial isolates

Morphological characteristic were studied for the isolated cultures by Gram's Staining. The morphological characteristics of isolated cultures are tabulated in **Table 3, Figure 1a-1c** and cultural characteristics are tabulated in **Table 4**.

Biochemical characterization of bacterial isolates

Biochemical characterization was done for idententification of bacterial isolates **(Table 5)**.

Isolated cultures were identified and conformed using traditional method which include culture characteristic on selective media, gram staining and biochemical reaction, according to Bergey's Manual of systematic Bacteriology.

Isolate 1: The species was gram positive, long rods in chain form and reacted positively to indole, methyl-red, nitrate reduction and urease test. The organism was negative to VP, catalase and oxidase. The organism does not utilized simmon's citrate. Hence isolate is *Bacillus sp*., **(Figure 2a)**.

Isolate 2: The species was gram negative short rods and reacted positively to VP, citrate, nitrate, and catalase and oxidase test. The organism was negative to indole, M-R and urease test. It produced pyocyanin pigment on agar plates. Hence isolate 2 is *Pesudomonas sp*., **(Figure 2b)**

Isolate 3: The species was gram positive, cocci in shape in cluster form and reacted positively to M-R, citrate, nitrate, catalase and oxidase test. The organism was negative to indole, VP and urease. The negative test resembles to *staphylococcus sp* **(Figure 2c)**.

Heavy metals Analysis

Pre-analysis of metals

Presence of the following heavy metals: mercury, arsenic, cadmium, lead, selenium, chromium and iron was checked. The analysis is summarized below in **Table 6**.

Mercury, arsenic, cadmium, lead and selenium was found Below the Detection Level. This indicates that waste contain only chromium and iron. Although iron was present in beyond the level and chromium is in range. So sample was treated with bacteria.

Biosorption of metals

The heavy metals reduced by Bacillus sp. at pH 6.9 – 7.0 and temperature 30 – 37˚C was listed below in **Table 7**. The chromium and iron were sorbed by *Bacillus sp*. The iron reduction was 60 % and chromium

reduction was recorded 75%. Temperature and pH varies

The heavy metals reduced by Pseudomonas sp. at pH 6.5 – 7.4 and temperature 30 – 37˚ C was listed below **Table 8**. The chromium reduction was recorded 75% and iron 45 %. Temperature and pH varies.

The heavy metals reduced by *Staphylococcus sp*. at pH 7.0 – 7.4 and temperature 37˚C was listed in **Table 9**.

Pot study

The different concentration of leachate effect shows on three plant varieties. All plants were grown on different concentration of leachate except Capsicum frutescens. The seedling cannot germinate in 100% concentration of leachate or cannot tolerate higher concentration of leachate. Excessive iron concentrations cause hindrance in plant growth. Both samples contain higher amount of iron, which result into no growth of plants. So treated leachate sample was taken for further study. The below tables shows root and shoot length with respect to various concentration.

The tabulated data **(Table 12)** depicts that the plants grew well when leachate is diluted with water or mixed with fertilizers and treated leachate plants shows growth with variation in shoot and root length. The sample collected from Anand **(sample 2)** is less toxic compared to Ahmedabad **(sample 1)**. Pictures of plants that grew well have been show for reference **(Figure 4.1, 4.2)**.

Table1: Physical characteristics of sample 1 and sample 2

S. No.	Parametres	Characteristics	
		Sample 1	Sample 2
1.	Color	Dark Brown	Greyish
2	Appearance	Liquid with suspended tiny particles	Liquid with residues of plant debris
3	Odor	Unfavourable	Unfavourable

Table 2: Chemical Characterization of Sample 1 and Sample 2

S. No.	Test parameters	Name of the instrument	Result	
			Sample 1	Sample 2
1	pH	pH meter	7.4	7.0
2	BOD_3	-	5830ppm	1560ppm
3	COD	-	21100ppm	42800
4	TOC	-	13.2	10
5	Nitrate	Spectrophotometer	30.52	19.05
6	Nitrite	Spectrophotometer	4.81	4.39
7	Ammonical Nitrogen	Spectrophotometer	440ppm	225ppm
8	Chromium	Spectrophotometer	6.98ppm	2.56ppm
9	Iron	Spectrophotometer	9.23ppm	3.23ppm
10	TS	Hot air oven	4500ppm	2500ppm
11	TSS	Hot air oven	16ppm	5.60ppm
12	TDS	Hot air oven	88960ppm	8090ppm
13	TON	-		

Table 3: Morphological Characteristic of bacterial isolates

Characteristics	Isolate – 1	Isolate – 2	Isolate – 3
Gram staining	Gram positive long rods	Gram negative short rods	Gram positive cocci

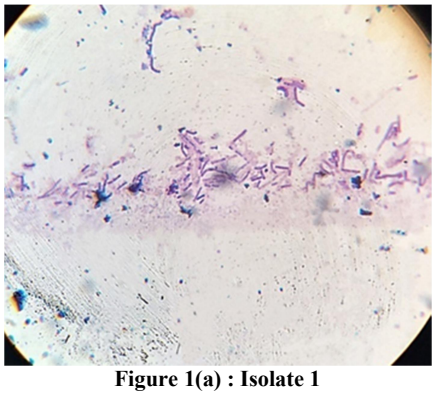

Figure 1(a) : Isolate 1

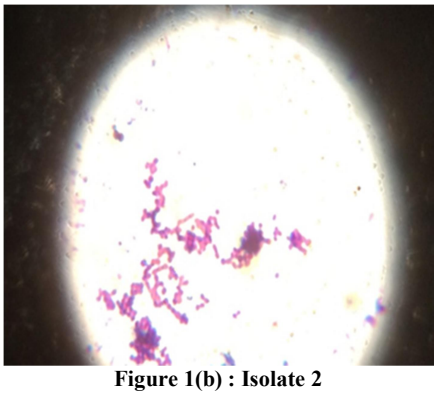

Figure 1(b) : Isolate 2

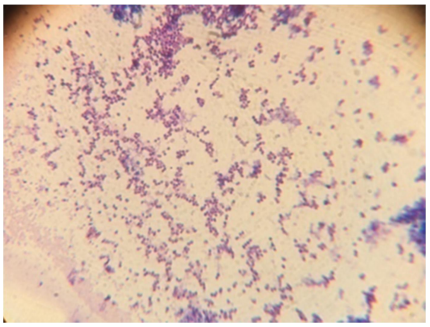

Figure 1(c) : Isolate 3

Table 4: Colony characteristic of bacterial isolates

Colony Characteristics	Isolate – 1	Isolate- 2	Isolate- 3
Size	Medium	Large	Punctiform
Shape	Irregular	Irregular	Circular
Texture	Dry	Smooth	Rough
Elevation	Flat	Raised	Convex
Margin	Lobate	Curled	Entire
Opacity	Translucent	Translucent	Translucent
Pigmentation	White	green	Reddish brown

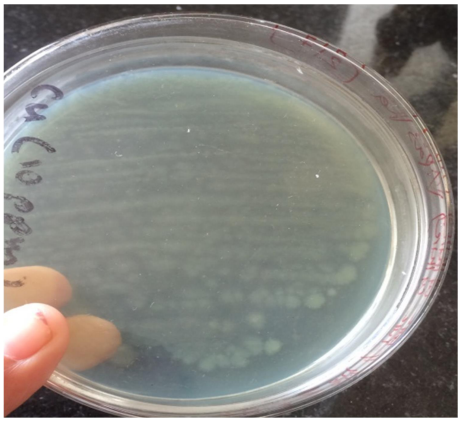

Figure 2(a) : Isolate 1

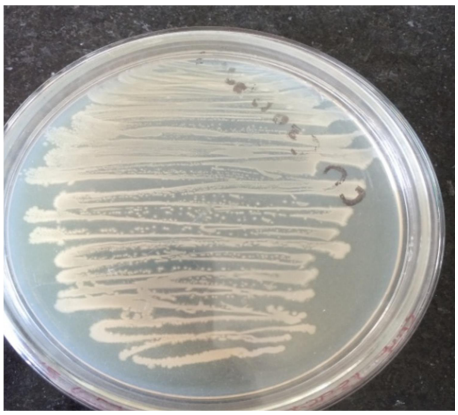

Figure 2(b) : Isolate 2

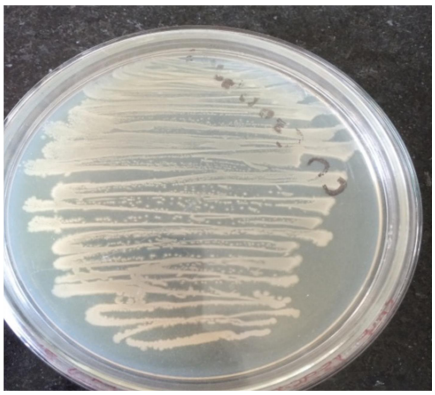

Figure 2(c) : Isolate 3

Table 5: Biochemical tests of Isolates

Biochemicals test	Isolate – 1	Isolate – 2	Isolate - 3
Indole test	+ve	-ve	-ve
Methyl – red test	+ve	-ve	+ve
Voges- Proskauer test	-ve	+ve	-ve
Citrate utilization test	-ve	+ve	+ve
Nitrate reduction test	+ve	+ve	+ve
Catalase test	-ve	+ve	+ve
Oxidase test	-ve	+ve	+ve
Urease test	+ve	-ve	-ve

("+ve" indicates positive test and "-ve" indicates negative test)

Table: 6 Heavy Metal Analysis by ICP – OES method (Pre – Analysis result)

S. No.	Heavy Metals	Instrument Detection Limit	Result (mg/l)	
			Sample 1	Sample 2
1	Mercury	0.0610	BDL	BDL
2	Arsenic	< 0.0530	BDL	BDL
3	Cadmium	0.0027	BDL	BDL
4	Lead	0.0420	BDL	BDL
5	Selenium	0.0750	BDL	BDL
6	Chromium	0.0071	1.6306	0.4015
7	Iron	0.0046	26.634	12.893

(BDL = Below Detection Level)

Table: 7 Biosorption of metals with *Bacillus sp*.,

S. No.	Site	pH	Temperature (˚C)	Cr (Before)	Cr (After)	Fe (Before)	Fe (After)
1	AMC	6.9	37˚C	1.630	0.286	26.634	14.857
2	Anand	7.0	30˚C	0.401	Not detected	12.893	8.857

Table: 8 Biosorption of metals with *Pseudomonas sp*.

S. No.	Site	pH	Temperature (℃)	Cr (Before)	Cr (After)	Fe (Before)	Fe (After)
1	AMC	7.0	37	1.630	1.246	26.234	21.121
2	Anand	7.2	37	0.401	Not detected	12.893	9.213

Table: 9 Biosorption of metals by Staphylococcus sp.

S. No.	Site	pH	Temperature (℃)	Cr (Before)	Cr (After)	Fe (Before)	Fe (After)
1	AMC	6.5	37	1.630	1.222	26.634	19.767
2	Anand	7.2	30	0.401	0.054	12.893	10.343

Table 10: Average root and shoot growth of *Cucumis sativus*

Samples	S. No.	Parameters	Leachate concentration (%)					
			Control	10%	20%	40%	Direct	Treated
Sample 1	1	Shoot length	24cm	10cm	22cm	30cm	No growth	25cm
	2	Root length	18cm	3.5cm	6cm	16cm	No growth	10cm
Sample 2	1	Shoot length	24cm	12cm	25cm	21cm	10cm	20cm
	2	Root length	18cm	5cm	10cm	10cm	1cm	12cm

Sample 1: AMC and Sample 2: Anand

Table 11: Average root and shoot growth of *Capsicum frutescens*

Samples	Sr. no	Parameters	Leachate concentration (%)					
			Control	10%	20%	40%	Direct	Treated
Sample 1	1	Shoot length	6cm	20cm	20cm	No growth	15cm	18cm
	2	Root length	10cm	16cm	10cm	No growth	5cm	11cm
Sample 2	1	Shoot length	6cm	No growth	5cm	2cm	2cm	No growth
	2	Root length	10cm	No growth	1cm	1-2cm	1cm	No growth

Table 12: Average root and shoot growth of *Solanum lycopersium*

Samples	S. No.	Parameters	Leachate concentration (%)					
			Control	10%	20%	40%	Direct	Treated
Sample 1	1	Shoot length	6cm	1.5cm	4.5cm	3cm	2cm	15cm
	2	Root length	12cm	5cm	10cm	12cm	1 cm	6cm
Sample 2	1	Shoot length	6cm	7cm	11cm	No growth	No growth	20cm
	2	Root length	12cm	3.5	5cm	No growth	No growth	12cm

Figure 4.1: Growth of Capsicum frutescens on different concentration of leachate and treated leachate

Figure 4.2: Growth of Cucumis sativus on different concentration of leachate

CONCLUSION

In the present study, a leachate was collected from two sites: AMC (Gyaspur landfill site) and Anand sewage site. From the results it can be concluded that the leachate sample collected from Anand had lower amount of COD, BOD, nitrate, nitrite and ammonical nitrogen compared to the leachate collected from AMC. Thus, the leachate from AMC had higher content of heavy metals. Biosorption assay was performed to treat the leachate samples which relatively showed decreased amount of heavy metals after the treatment of leachate. The microbes play a vital role in the remediation of heavy metals and they have high tolerance against heavy metals. The result showed that Bacillus sp. reduced heavy metals more than other microbes. Pot studies were carried out using leachate samples; that was, without treatment and after the treatment of leachate. Thus, good growth of plants was observed for treated leachate samples.

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